Simultaneous Effects of Metformin and Sitagliptin on the Contents of Insulin Resistance Proteins Glucose Transporter 4 and Protein Kinase B in Diabetic Patients' Adipose Tissue

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Abstract

Objective: Obesity is a factor in the development of insulin resistance and type 2 diabetes**.** Obesity contributes a wide variety of metabolic changes such as insulin resistance. The insulin signal mechanism to intra-cells occurs in insulin resistance, primarily in adipose tissue cells, which can be appropriate targets for therapeutic approaches by recognizing the proteins in this pathway. The study aimed to evaluate the simultaneous impact of metformin and sitagliptin on the expression of protein levels involved in insulin resistance Protein Kinase B (Akt) and Glucose Transporter 4 (GLUT4) in diabetic adipose tissue.

Materials and Methods: In order to evaluate the content of proteins involved in insulin resistance Akt and GLUT4 in adipose tissue of diabetic patients with the use of SDS-PAGE and western blot analyses, we studied 6 persons of type 2 diabetic patients who obtained 3 months of care with simultaneous metformin and sitagliptin, 4 persons returned from them after treatment and 8 persons as a stable case (control group).

Results: There was an increase in glucose intake and a decrease in serum glucose levels (*P*-value= 0.025) and no decrease in insulin resistance (*P*-value= 0.6) following simultaneous metformin and sitagliptin therapy, but no improvement in serum insulin levels (*P*-value=1.01). Increases in the content of Akt protein (*P*-value= 0.682) and GLUT4 protein (*P*-value= 0.851) involved in insulin resistance in diabetic patients' adipose tissue, were not observed.

Conclusion: Simultaneous treatment with metformin and sitagliptin had no effect on insulin resistance proteins Akt and GLUT4 in type 2 diabetic adipose tissue.

Keywords: Insulin Resistance, Metformin, Sitagliptin, Type 2 diabetes, Glucose transporter 4, Protein Kinase B

Introduction

Obesity is a significant risk factor for the development of type II diabetes (T2DM) and insulin resistance, and the metabolic changes such as insulin resistance. Insulin resistance has changed the mechanism of an insulin signaling pathway to

intra-cells, especially adipose tissue cells, which can be a good target for therapeutic strategies by recognizing the proteins in this pathway (1). Insulin changes the conformation of its receptor and phosphorylates and activates it by binding to its receptor (insulin receptor, IR) in the membrane of adipocytes. Then, in the cytosol of these cells, IR activates another protein called Insulin-like Receptor Substrate-1 (IRS-1), which in turn activates another protein called kinase phosphatidylinositol 3 (PI3K), that in turn protein kinase B (Akt), that in turn activates another protein called Mammalian Target Of Rapamycin (mTOR).

The glucose transporter 4 (GLUT4) protein in the cytosol of the two cell types, muscle cells and adipocytes, allows GLUT4 to migrate to the membrane surface of these cells and makes GLUT4 even more involved, rendering mTOR in the cytosol a multi-purpose protein (2). The more glucose enters these cells through increased GLUT4 in the cell membrane, the more glucose is absorbed.

This route is impaired in diabetics with obesity, particularly T2DM, and glucose enters these cells in distress. Therefore, diabetic patients need to adjust their care lifestyle so that high levels of glucose will affect their body tissues as a toxic drug (3).

Dependent on the therapeutic approaches of American Diabetes Association (3), Metformin can aid in care of T2DM patients by improving their diet, weight management, physical activity, and exercise and preparation with the intention of treatment and the use of a single drug.

If these are followed and after 3 months in T2DM patient is carried HbA1c test and not increase HbA1c levels, therapeutic actions have been effective but if HbA1c is not only not decreased, but increased, the second line of therapeutic actions is required, patients will receive two drugs, metformin and another drug, such as sulfonylurea, a thiazolidinedione, dipeptidyl peptidase inhibitors (such as sitagliptin), and Insulin infusion are indicated.

If HbA1c is measured again and the HbA1c level is low, diabetes is well controlled in these patients, but if HbA1c is high, diabetes is not controlled in these patients and the need for action in the third stage and the use of three or more drug treatments (2). Metformin and sitagliptin are commonly used as therapeutic purposes in T2DM. Metformin is a biguanide. Its main mechanism in liver cells decreases gluconeogenesis by reducing glucose synthesis. Metformin increases the sensitivity of cells to insulin, so that insulin receptors go to the membrane surface of the cells and also increase the expression of these receptors. Also, insulin binding to its go to the membrane surface of the cells and also increases the expression of these receptors. In T2DM, insulin binding to its receptors also decreases insulin resistance. On the other hand, metformin causes glucose uptake by cells via GLUT4 (3). Sitagliptin is one of Dipeptidyl peptidase 4 Dipeptidyl peptidase 4 (DPP4) enzyme inhibitor medicine group. This enzyme is in the pancreatic islets of Langerhans. The enzyme breaks down and destroys two compounds called glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic peptides (GIP). GLP-1 and GIP in beta cells produce and secrete insulin from them. Sitagliptin is an inhibitor of the DPP4 enzyme, which inhibits the duration of the decomposition of both GLP-1 and GIP compounds, and the longer the two compounds remain in beta cells, and more insulin is produced and secreted (4). The mouse models resulted these two drugs were effective on the mechanism of insulin action and be able to eliminate insulin resistance by improving and eliminating the problems of the insulin signaling pathway. Human studies of the insulin signal transform pathway and mechanism however are incomplete. Drugs have a low impact in adipose tissue and are only used infrequently (5).

The current research aims to demonstrate improvements in insulin resistance in T2DM insulin signal transform pathway in adipose tissue, as well as the effects of two

medications, metformin and sitagliptin, on adipocyte cells of human adipose tissue, as well as the use of techniques and laboratory methods to evaluate the insulin signaling pathway in adipose tissue and increase its rate.

Materials and Methods

We studied 6 T2DM patients in Yazd Diabetes Research Center (from 2017 to 2019 Yazd University of Medical Sciences-Department of Biochemistry) who were treated with Simultaneity sitagliptin and metformin. Four patients returned after treatment (persons who have not yet taken medication have recently been diagnosed with a blood glucose test, the so-called New Case test, and diabetic patients who are taking medication, taking pills or taking insulin have been eliminated) and 8 persons as the healthy case (control group). Participants in this study were randomized to receive simultaneity metformin and sitagliptin.

Getting Blood sample and calculation of Homeostatic model assessment-Insulin Resistance (HOMA-IR)

After the justification of each patient, 10 ml of blood was taken in a tube without anticoagulant before treatment and serum were isolated. The glucose, lipid profile, and insulin profile of these individuals were measured. Insulin resistance (IR) of participants was measured based on the HOMA-IR formula (6). A method used to quantify insulin resistance (IR) and beta-cell function is homeostatic model evaluation (HOMA). It was first described by Matthews et al, under the name HOMA (7). HOMA-IR is equal to blood glucose (mg%) × blood insulin (μ U/ml) / 405, both glucose and insulin are during fasting. Patients with insulin resistance greater than 2 were considered as insulin resistant and continued this study. Separated serum was stored at -80 °C.

Abdominal subcutaneous adipose tissue sampling

By expert physician and surgeon, all groups underwent a subcutaneous adipose tissue test according to common methods (8,9). To remove the fat tissue sample from abdominal subcutaneous, the participants were first locally anesthetized. The surgeon then removed the subcutaneous adipose tissue with a scalpel and surgical scissor for each participant by cutting in the anesthetized abdominal area.

Processing adipose tissue and preparation cellular extract

An adipose tissue sample from participants was entered into a 50-millimeter falcon tube containing a transfer medium (saline 9%, phosphate-buffered saline (PBS) or 199 M199 environments from Gibco, with room temperature glutamine and penicillin 100 U / L and streptomycin 100 μ g / ml and gentamicin 50 μ g / ml total). We processed adipose tissue samples from each participant to separate fatty adipocytes. We performed all of the following under the laminar hood in a sterile state. To do this, we minced adipose tissue sample in the plate with very sharp sterile scissors into smaller pieces of 1-2 cubic centimeters until a papescent texture is reached, then put a sterile laboratory hopper into which we placed a filter and placed the funnel on a falcon tube; a tissue sample. We added the sliced fat to the filtered funnel, then added to the saline or PBS hopper at room temperature to remove the fat and red blood cell counts. This specimen was then weighed. To isolate the adipocytes, we poured the minced tissue sample from the previous stage into a 50-milliliter falcon tube and poured 3 ml of collagenase solution (1 gr collagenase powder in 1 ml solution) per 1 gram of tissue into the tube. We placed the tube in a 37 °C shaking at 100 rpm for 90 minutes to find a watery consistency mixture. We rotated the tubes slowly every 15 minutes and observed the degree of digestion. The resulting mixture was filtered under a laminar hood using cell culture media to separate the adipocyte cells from the cell residues. Adipocytes were then suspended into DMEM: F12 medium. Then, to each T2DM patients for a three-month therapy

period, a metformin and sitagliptin pills were given. After 3 months, diabetic patients returned to the Yazd center of research-therapy diabetes. After treatment, Blood samples and subcutaneous adipose tissue were taken from patients such as before treatment. At this stage, intended tests were performed on the patient's serum and were carried all of the processes on the adipose tissue samples of patients, as in the before treatment stage (cellular extract of the adipose tissue stored at -80 °C).

Performance of SDS-PAGE and Western Blot

The proteins GLUT4 and Akt in the cellular extract of the adipose tissue samples participants were measured by SDS-PAGE and Western Blot methods with use from mouse monoclonal antibody (Santa Cruz Biotechnology, INC) in each intended protein.

Statistical analysis

We assessed statistically different rates among intended groups with t-test and one-way ANOVA in SPSS version 17 and Graphpad Prism 7.04 software, respectively. Protein bands were analyzed by IMAGE J software. Significance level with 95% confidence and 80% power was considered to observe 0.15 differences between groups.

Ethical considerations

This study was approved by the ethics committee of Shahid Sadoughi University of Medical Sciences, Yazd, Iran. (Ethics Code: Code IR.SSU.MEDICINE.REC.1396.54)

Results

We briefed clinical specifications in table 1. Thereafter, simultaneous treatment with metformin and sitagliptin, fasting blood glucose levels decreased after vs. before treatment (*P*-value= 0.025), but insulin (*P*-value= 1.01) and HOMA-IR levels did not decrease (*P*-value= 0.6) in T2DM patients compared to control groups, and decreased levels of HbA1c, triglycerides, total cholesterol, and LDL-cholesterol after treatment vs. before treatment, respectively. Insulin resistance decreased after treatment compared to before treatment, which was higher compared to the control group.

As shown in figure 1, there was not a statistically significant difference in the relative contents of GLUT4 before treatment with metformin-sitagliptin compared to the control group (*P*-value= 0.9), also not after treatment (*P*-value= 0.851).

There was not statistically significant difference in the relative contents of Akt before treatment with metformin-sitagliptin compared to the control group (*P*-value= 0.763). Moreover, there was not a statistically significant difference in relative contents Akt after treatment compared to before treatment (*P*-value= 0.682).

Table 1. Medical specifications of patients with diabetes before and after simultaneous treatment with metformin and sitagliptin

Specifications	Diabetic patients		*P*-value	Controls (n=8)	*P*-value
	Before Treatment (n=6)	After Treatment (n=4)			
Age (Years)	44 (±4)	47 (±5)		40 (±5)	
BMI (Kg/m^2)	33 (±3)□†	33 (±4)□	0.77□	27 (±1)†	0.03†
Blood glucose (mg / dl)	219 (±39)□†	150 (±29)□	0.028□	120 (±25)†	0.025†
Blood insulin (µ U / ml)	10.9 (±2.2)□†	12.3 (±1.5)□	1.01□	4.2 (±0.1)†	0.02†
HOMA-IR	5.2 (±0.7)□†	4.4 (±0.7)□	0.6□	1.4 (±0.6)†	0.031†
Serum triacylglycerol (mg / dl)	264 (±67)□†	245 (±47)□	0.571□	145 (±25)†	0.023†
Blood cholesterol (mg / dl)	228 (±23)□†	216 (±15)□	0.613□	170 (±13)†	
LDL-c (mg / dl)	134 (±14)□†	122 (±17)□	0.031□	106 (±12)†	0.02†
HDL-c (mg / dl)	42 (±4)□†	45 (±4)□	1.07□	35 (±4)†	0.027†
HbA1c (%)	7.9 (±0.7)□†	6.4 (±0.5)□	0.046□	6.3 (±1.1)†	0.037†

Values were mean ± SEM
□ Before treatment vs after treatment
† Before treatment vs Controls

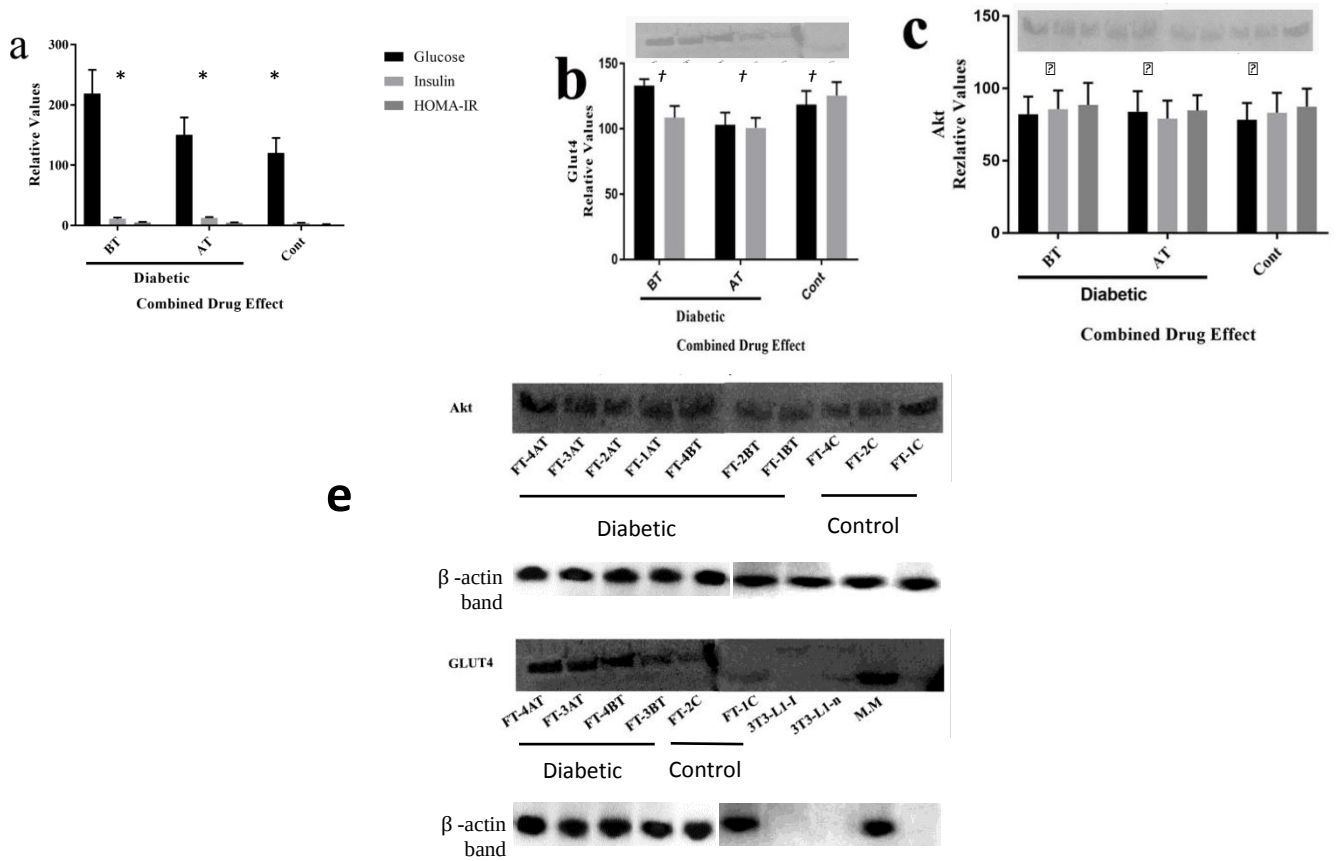

Figure 1

Effects of simultaneity metformin and sitagliptin treatment on serum glucose and insulin levels in diabetic individuals in comparison with control group(*P-value<0.05)[a], effects of simultaneity metformin and sitagliptin treatment on GLUT4 relative values in adipose tissue diabetic individuals in comparison with control group(†P-value > 0.05)[b], effects of simultaneity metformin and sitagliptin treatment on Akt relative values in adipose tissue diabetic individuals in comparison with control group (□P-value > 0.05)[c], Western blot analyses of: Glucose Transporter 4(GLUT4); Protein Kinase B(Akt). Note that: (1) samples within western blot analyses routinely contained (or were prepared from) equal amounts of lysate protein, (2) no significant changes were seen in levels of indicated signaling proteins in adipose tissue of diabetic vs control participants or in diabetic adipose tissue after vs before treatment. Accordingly, it may be inferred that the levels of phosphorylated proteins in immunoblots reflected alterations to the phosphorylation status of a given amount of the signaling protein, rather than a change in the amount of signaling protein. Values are mean±SEM

BT:Before Treatment, AT: fter Treatment, Cont or C: Control, M.M: Mouse Muscle

Discussion

In our study, the simultaneity of treatment with metformin and sitagliptin was not statistically relevant in the insulin signaling protein resistance needed in the adipose tissue insulin pathway, i.e. GLUT4, Akt in diabetic patients' adipose tissue.

In other researches, when thiazolidinedione or metformin was used alone, comparatively significant and widespread changes in the inactivation of these signaling factors were not apparent and clamp studies used full insulin stimulation (10,11).

In another study, combined thiazolidinedione-metformin therapy improved insulin signaling to T2DM muscle insulin receptor, IR

Substrate-1/PI3 Kinase, Protein Kinase C, and protein Kinase B at sub-maximal and maximal levels. (12).

In another study, diabetes patients who received initial combination treatment with sitagliptin-metformin and drug-alone single therapy saw major and sustained improvements in glycemic regulation (*P*-value= 0.05) and were well tolerated for 104 weeks (13). The best outcomes have been obtained with tizanidine and sitagliptin as monotherapy or as dual therapy, combining partial PPAR-γ agonism and PPAR-alpha activation with sustained incretin activity within the liver (5).

It should be noted that the changes seen in diabetic adipose tissue after concurrent treatment with metformin and sitagliptin are likely to be secondary to primary changes in muscle and liver tissue, as both medicines are known to cause multiple effects that may have a positive impact on adipose tissue insulin signaling. In this respect, the prevalence of reparations in all the signaling proteins examined may largely indicate the insulin resistance reparations recorded in the present study.

Finally, It was important that to find serum insulin contents decreased meaningfully after treatment with simultaneous metformin-sitagliptin, since very high insulin may stimulate increases in the hepatic lipid product (14) and as a result, T2DM with atherosclerosis can benefit from this treatment. In blood glucose, triglyceride, cholesterol total, and LDL-c, beneficial descending procedures were too perceived, however in our study, repair of HDL-cholesterol was not evident. As a result, improvements in parameters and other metabolic variables, as well as clinical consequences, should be monitored in enduring research of sitagliptin-metformin treatment. Moreover, the substantial repair of insulin signaling mechanisms in diabetic adipose tissue as well as metabolic variables observed after short-term concurrent treatment with metformin and sitagliptin at test to their suitability for the treatment of T2DM, especially in the first stages disease.

Conclusions

Finally, the simultaneity use metformin-sitagliptin with the desired more than three-month treatment period, in addition to decreasing blood glucose and other parameters in the blood but not reducing insulin, also failed to promote and increase the key proteins in insulin signaling.

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Conflict of Interest

Authors of this article have not conflict of interest

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